

In a coassigned U.S. patent application Serial Number 09/357,440, filed July 20, 1999. The use of a centrifuge is disclosed to reduce non-specific fluid surface effects. In this case, a reaction cell is agitated about an axis along the centrifugal force. While this approach 5 offers certain improvements in the amount of sample required for hybridization and in hybridization rates, further advances are desired. What is needed is a system and method that provides for faster hybridization and smaller sample volumes.

#### SUMMARY OF THE INVENTION

10 The present invention facilitates solid-phase chemical, e.g., affinity, reactions as of a sample liquid to a probe array by agitating the sample liquid while under the influence of a centrifugal force greater than 1G, the gravitation force at Earth's surface. The supergravity centrifugal force is more effective than normal gravity in 15 overcoming the resistance imposed by liquid viscosity and non-specific binding forces to liquid flow. By permitting faster liquid flow, the invention can achieve higher replenishment rates and thus faster hybridization.

20 Preferably, the agitation involves rotation about an agitation axis that is more orthogonal to than along the centrifugal force. More specifically, the agitation axis can be more parallel than not (i.e., deviates at most 45° from parallel) to the centrifuge axis. If the agitation axis is along or nearly along the centrifugal force, the sample liquid churns in circles parallel to the array. The preferred agitation 25 axes provide for tidal (i.e., periodic ebb and flow) motion of the sample liquid in which much of the array is alternately covered only by a thin liquid film and then replenished by a thick wave of sample liquid.

30 Also, preferably, the array is two-dimensional is more orthogonal than not to the centrifugal force. When the array is orthogonal to the centrifugal force, the centrifugal force urges sample liquid against the array. Of course, the agitation involves deviations

from orthogonality, and unless the array is appropriately curved, the centrifugal force is not orthogonal to the array everywhere on the array. Nonetheless, averaged over time and the array, the centrifugal force is preferably nearly orthogonal to the array.

5        In a preferred realization of the invention, the two-dimensional array is (on average) orthogonal to the centrifugal force and the agitation axis is parallel to the centrifuge axis. The agitation involves periodic reversals in the direction of agitation rotation, further emulating tidal action. The centrifuge period (corresponding to a 360°  
10 rotation of a centrifuge rotor) is preferably greater, and typically much greater, than the agitation period (which includes two reversals of agitation direction). In analogy with gravity (instead of centrifugally) driven motion, the array can be considered as rocking teeter-totter fashion about a position parallel to Earth's surface. In this realization,  
15 once hybridization is completed, the reaction cell (and thus the probe array) can be rotated, e.g., inverted, about the agitation axis for spin drying using centrifugal action.

          A major advantage of the present invention is that it provides for a relatively high replenishment rate, as well as a relatively effective  
20 tidal replenishment motion. Thus, the invention can provide for rapid hybridization. In addition, the invention can make efficient use of sample liquid, which is usually costly, time-consuming to produce and/or of limited availability. These and other features and advantages of the invention are apparent from the description below  
25 with reference to the following drawings.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic plan view of an array hybridization system with a first set of reaction cells in accordance with the present invention.

5 FIGURES 2A-2C show three orientations of a reaction cell of FIG. 1 being agitated in accordance with the present invention by the system of FIG. 1.

FIGURE 3 shows a fourth orientation of the reaction cell of FIGS. 2A-2C.

10 FIGURE 4 shows the array hybridization system of FIG. 1 with a second set of reaction cells.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

An array-hybridization system AP1 in accordance with the present invention is shown in FIG. 1. System AP1 comprises a centrifuge subsystem 10 and an agitation subsystem 20. Centrifuge subsystem 10 includes a centrifuge motor 11, a centrifuge drive chain 13, a centrifuge drive shaft 15, and a centrifuge rotor or "turntable" 17. Agitation subsystem 20 includes an agitation-drive motor 21, an agitation-drive chain 23, an agitation-drive shaft 25, an agitation-drive gear 27, and three agitation-drive mounts 29. Agitation-drive mounts 29 are rotatably coupled to turntable 17. Each agitation-drive mount 29 holds a reaction cell 30.

Reactions cells 30 are similar so the following description of any one is representative. A reaction cell 30 includes a substrate 31 and a cover 33 so as to define a 2cm x 2cm x 0.25 mm interior volume or "reaction chamber" 35. (In the figures, the thickness of reaction cell 30 is exaggerated for clarity.) During hybridization, this interior volume is partially filled with sample liquid 39, with the remainder of the cell interior volume being filled with gas 37, e.g., dry air or

nitrogen. A hybridization array 40 with 10,000 probes arranged in a 100 x 100 array is defined on substrate 31 on the side contacted by sample liquid. Two septa (not shown) in each cover 33 permit fluid to be introduced and removed from reaction cell 30.

5        Motors 11 and 21, which are both servo motors, are located below turntable 17. Agitation drive shaft 25 and centrifuge drive shaft 15 are coaxial, with agitation drive shaft 25 extending though a hollow centrifuge shaft 15. Centrifuge drive shaft 15 is rigidly coupled to turntable 17. Agitation drive shaft 25 is rigidly coupled to agitation 10 drive gear 27, which is engaged with the teeth of mounts 29.

Mounts 29 are rotatably coupled to turntable 17 about respective agitation axes 41. Each agitation axis 41 extends parallel to a centrifuge axis 43 (through shafts 15 and 25). Each agitation axis 41 extends though a respective reaction cell, nearly bisecting the 15 respective hybridization array 40. Each agitation axis 41 is spaced 10 cm from the centrifuge axis 41.

Centrifuge motor 11 determines the rotation rate of turntable 17. The gear ratio of centrifuge drive motor to turntable drive shaft 15 is 1:1, so that the rotation rate of turntable 17 is the 20 same as the rotation rate for motor 11. The gear ratio of agitation drive motor 21 to agitation drive shaft is 2:1, and the gear ratio of mount 29 to agitation drive gear 27 is 3:1. When there is no agitation, agitation motor 21 rotates at exactly half the rate of centrifuge motor 11. To effect agitation, the rotation rate of agitation motor 21 25 is increased and decreased in a controlled manner so that it alternately leads and lags centrifuge motor 11 in phase. Both motors 11 and 21 are driven cooperatively by a servo-controlled unit such as manufactured by Galil in Sunnyvale, California. Alternatively, the invention provides for other means for achieving the described 30 agitation and centifugation.

Generally, centrifugal forces much greater than the local gravitational field are desired to overcome non-specific liquid surface binding forces. Turntable 17 can be rotated at 1000 revolutions per minute (RPM), to achieve a centrifugal force of 112G. Much higher forces are readily achievable. For example, at 3000 RPM, a centrifugal force of 1004G is achieved.

The agitation amplitude is selected to be about  $\pm 6^\circ$  to effect full "sloshing" of the sample liquid. The agitation rate depends on the sample liquid and the centrifugal force. A typical value would be a 5 Hz agitation, which would yield ten replenishments per second.

In FIG. 2A, reaction cell 30 is shown titled counterclockwise  $+6^\circ$  relative to a central centrifugal force vector 45 at the beginning of an agitation cycle. (Agitation angles are exaggerated in FIGS. 2A-2C for clarity.) In this orientation, all sample liquid 39, other than a thin film, is at the end 47 shown to the left in FIGS. 2A-2C. The surface of sample liquid 39 in the static state represents a constant radius from centrifugal axis 43 (FIG. 1).

In FIG. 2B, reaction cell 30 has rotated clockwise past a level (orthogonal to a centrifugal force vector 45) orientation to a  $-2^\circ$  clockwise orientation. In this orientation, some of the liquid has reached the opposite end 49 (to the right in FIGS. 2A-2C). Most of the remaining liquid is still at the clockwise end 47, while a tapered sheet of sample liquid 39 extends between the ends 47 and 49.

In FIG. 2C, reaction cell 30 has rotated to an extreme clockwise position at  $-6^\circ$ . In this position, except for a thin film, sample liquid 39 is at the right end 49 of reaction cell 30. This completes the first half of an agitation cycle. The second half of the agitation cycle begins with the orientation of FIG. 2C and ends with the orientation of FIG. 2A. An intermediate orientation of  $+ 2^\circ$  (counterclockwise) for the second half is shown in FIG. 3.

In FIG. 3, reaction cell 30 is being rotated counterclockwise as indicated by arrows 50. Centrifugal force arrows 45 are shown broken into a component 51 along array 40 and a component 53 orthogonal to array 40. The component 51 along array 40 urges sample liquid 39 toward (left) end 47. This movement is indicated by arrows 55, which show a tapered liquid profile flowing toward (left) end 47. A curved arrow 47 shows a return motion for liquid sample 39. This return motion provides for highly desirably vertical mixing.

The vertical mixing assures that every target molecule spends some time close enough to array 40 for binding to occur. The centrifugal force 45 helps overcome the inertia of the liquid and its non-specific binding forces with the substrate so that a high agitation rate can be maintained. The advantages of the invention can be understood with the following, admittedly approximate, understanding of the hybridization process.

When the agitation rate is doubled, each target molecule is likely to be found half as far from a respective probe for half the time. When it is half as far, it is four times as likely to hybridize. However, the interval over which it can hybridize is half as long. Thus, in principle, doubling the agitation rate doubles the hybridization rate. This linear relationship applies until non-specific binding fluid forces prevent sample liquid from completing its motion across the array. The stronger the centrifugal force, the higher the agitation rate can be raised before this limiting consideration applies. Thus, the centrifuge rate can be increased until the forces involved adversely affect specific binding or threaten the integrity of the hybridized or non-hybridized species.

In FIG. 1, the agitation axes are parallel to the centrifuge axis and the hybridization arrays are generally orthogonal to the centrifugal force. In other embodiments, the hybridization arrays are also generally orthogonal to the centrifugal force, but the agitation